

Rheological Properties and Injectability of β -Tricalcium Phosphate-Hyaluronic acid/Polyethylene Glycol Composites Used for the Treatment of Vesicourethral Reflux

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Abstract

Beta-tricalcium phosphate pastes were prepared by combination of water and hyaluronic acid or polyethylene glycol at powder to liquid ratio of 1.4 g/mL. The effect of adding hyaluronic acid and polyethylene glycol on thixotropy, viscosity and injectability of the pastes was measured. According to the results, hyaluronic acid caused to increase in both viscosity and thixotropy of paste more than polyethylene glycol. The lower area of thixotropy loop indicated that the rate of structure rebuilding was increased at the presence of polyethylene glycol. In contrast to hyaluronic acid or polyethylene glycol-added samples, the paste prepared with distilled water was difficult to be injected because of the filter-pressing phenomenon though it had lower viscosity than that of other ones.

Keywords

Beta-Tricalcium Phosphate; Hyaluronic Acid; Polyethylene Glycol; Injectability; Rheological Properties

Introduction

Beta-tricalcium phosphate (β -TCP) is widely used as bone substitute in the form of powder, granule or block. A major disadvantage of preformed scaffolds is that the surgeon needs to machine the graft to the desired shape or carve the surgical site around the implant to fit in a bone cavity. This leads to an increase in bone loss, prolonged surgical time and trauma of the surrounding tissue. With the development of noninvasive approaches such as percutaneous surgery, injectable biomaterials will be needed. Injectable biomaterials such as calcium phosphate (CP) pastes, shorten the surgical operation time, minimize damaging of large muscle retraction, reduce postoperative pain and scar size, and achieve rapid recovery.

Calcium phosphate pastes are appropriate synthetic

bone substitute in osseous surgery and increasingly used in minimal invasive surgery for repairing bone defects. Augmentation of femoral neck fracture, defects of distal end of the radius and the fracture of the spine are examples of CP hard tissue applications. Injectable calcium phosphate pastes are also useful for soft tissue treatment e.g. modification of urethra in vesicourethral reflux disease. For both applications, injectability and flow properties are important because CP paste should pass through a narrow and relatively long path (~50 cm) to be delivered into defect site. In vesicourethral treatment, CP paste is transferred to urethra-bladder neck through a catheter to produce an excess volume.

Several strategies have been used to modify the flowability of CP pastes using various additives without significant changes in other properties. For instance, improved injectability of calcium phosphate paste has been obtained using polysaccharides, sodium glycerophosphate, lactic acid, glycerol, chitosan and polyvinyl alcohol (PVA) Sarda et al. were also found that the injectability of α -tricalcium phosphate/water mixture was increased by 50–100% using citrate ions.

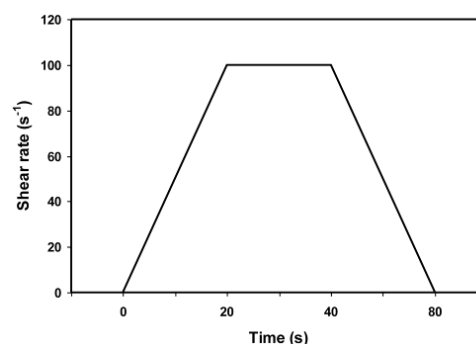


FIGURE 1. PROGRAM FOR THE THIXOTROPY AND VISCOSITY TESTS

However, limited studies have been done on rheological properties of calcium phosphate pastes containing additives to improve injectability. In this study, the influence of hyaluronic acid and polyethylene glycol (PEG) on thixotropy, viscosity and injectability of β -tricalcium phosphate pastes has been investigated which can be used for the treatment of vesicoureteral reflux disease.

Materials and Methods

Starting Materials and Characterization

β -TCP was synthesized through solid-state reaction between calcium carbonate (Merck, Germany) and dicalcium phosphate (Merck, Germany) reagents. Briefly, these materials were mixed together in Ca/P molar ratio of 1.5 in ethanol medium by a planetary mill. The mixture was dried at 70°C for 24 h, manually grounded in an agate mortar with a pestle and passed through a 100 mesh sieve. The obtained powder was calcinated at 950°C for 3 h.

The phase composition of β -TCP was checked by an automated X-ray diffractometer (XRD) (Philips PW3710) with Cu-K α radiation. The surface area of synthesized β -TCP was measured by nitrogen adsorption technique known as Brunauer, Emmel and Teller (BET) method (Micrometrics, Gemini). The particle size was obtained by Laser particle size analyzer, Fritsch Analysette 22, in ethanol medium. For the liquid phase of pastes, the following materials were used: hyaluronic acid (Jinan Haohua Industry, China), acetic acid (Merck 100062, Germany), polyethylene glycol 400 (Merck, Germany) and distilled water. 3 wt% hyaluronic acid solution was prepared in 1 wt% acetic acid solution.

TABLE 1. CHEMICAL FORMULA OF DIFFERENT β -TCP PASTES INVESTIGATED IN THIS STUDY

sample code	Polyethylene Glycol (Vol.%)	hyaluronic acid solution (Vol.%)	distilled water (Vol.%)
W100	0	0	100
W50H50	0	50	50
W50P50	50	0	50
W75P25	25	0	75

Various pastes were made by mixing β -TCP powder and different liquid phases (powder to liquid ratio (P/L) = 1.4 g/mL) according to table 1.

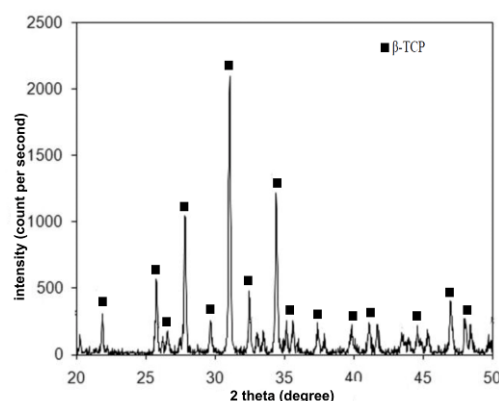


FIGURE 2. THE XRD PATTERN OF SYNTHESIZED B-TCP

Rheological Properties and Injectability

The rheological properties were examined by using a controlled-rate rotational rheometer (MC R301) with a coaxial cylinder system. The as-prepared paste was poured into the rheometer and tests were taken under shear rate-controlled mode. The program used for the rheological investigations is shown in Figure 1.

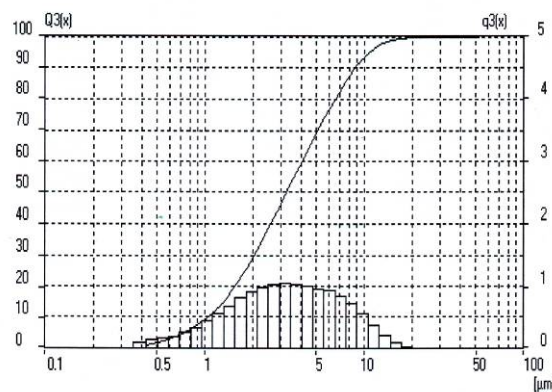


FIGURE 3. PARTICLE SIZE DISTRIBUTION OF SYNTHESIZED B-TCP

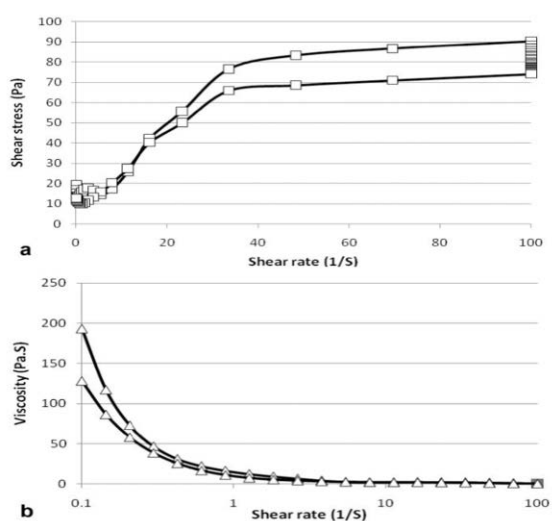


FIGURE 4. THIXOTROPY (A) AND VISCOSITY (B) CURVES OF W100 PASTE

For injectability measurements, the paste was transferred into a 1 mL syringe with a tip diameter of 2 mm. The paste was extruded by a compressive load vertically mounted on top of the plunger using a universal testing machine (Zwick/Roell –HCR 25/400) at a crosshead speed of 1 mm/s and the applied force was plotted against the plunger displacement.

Result and Discussion

Figure 2 shows the X-ray diffraction diagram of the synthesized β -TCP powder. All peaks belonged to whitlockite phase (ICDD 009-0169) and no impurity phase was detected.

The mean particle size and specific surface area of β -TCP particles were 3.2 μm and 56.9 m^2/g , respectively. A graph that shows the change of particle size versus volume of particles is provided in figure 3.

Figures 4-7 show the thixotropy and viscosity curves of the pastes made of β -TCP and different liquids. For the paste prepared with distilled water (Fig. 4), hysteresis loops are observed for both shear stress vs. shear rate and viscosity vs. shear rate curves. For both upward and downward curves, shear stress and viscosity depended strongly on the shear rate. The dependence of viscosity to shear rate is considerable for shear rate of 0 to 1 s^{-1} . On the other hand, it remains constant at shear rates higher than 1 s^{-1} . A nearly constant shear stress is also observed for shear rate of 40 to 100 s^{-1} , while for shear rate of 0-40 s^{-1} upward and downward curves change linearly.

As shown in Figure 5, the yield stress and viscosity of the paste increases by adding hyaluronic acid solution. The thixotropy loop area of the paste made of hyaluronic acid solution and β -TCP powder is wider than the paste prepared with distilled water.

Thixotropy loop is an important factor for the description of paste stability i.e. the larger thixotropy loop is, the more stable paste is. Thus the addition of hyaluronic acid solution increases stability of β -TCP paste.

Hyaluronic acid is a polysaccharide polymer with long chains that have strong interaction with calcium phosphate particles to form secondary bonds with them. The formed internal structures causing thixotropy and increased viscosity are disturbed by the application of shear stress that leads to diminished viscosity. Hyaluronic acid is capable to absorb and

preserve water and thus preventing CP particles to be washed out. The presence of a viscous polymeric layer around CP particles provides a proper environment for slipping during applying shear stress [12].

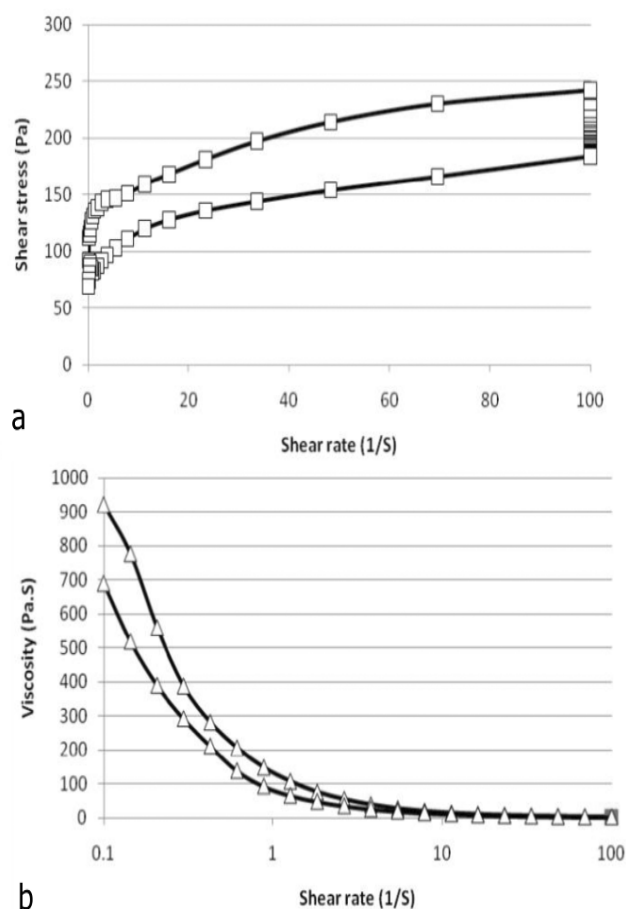


FIGURE 5. THIXOTROPY (A) AND VISCOSITY (B) CURVES OF W50H50 PASTE

By adding 25 and 50 vol. % PEG to the paste, a notable increase in viscosity has been observed (compared to paste made of pure water) (Figs. 6, 7). This phenomenon can be described by the microstructure of the paste. It is suggested that a network is formed through hydrogen bonding between PEG chains, leading to the production of many colloidal particles which can join together through the Vander Waals interactions and form spatial net structure, and further strengthen the network of the paste [13]. Additionally, at the presence of PEG, the values of viscosity in upward and downward curves are close to each other, which means that the rate of structure rebuilding in PEG-containing paste is higher than that of hyaluronic acid-added one. Although hyaluronic acid and PEG additives increased thixotropy, they lower friction between CP particles and facilitate injection process as shown in next section.

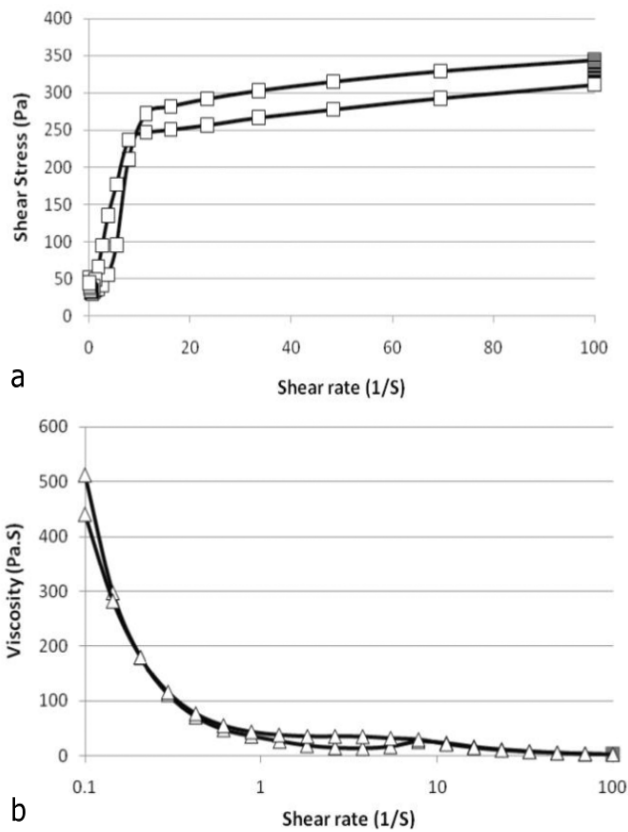


FIGURE 6. THIXOROPY (A) AND VISCOSITY (B) CURVES OF W50P50 PASTE

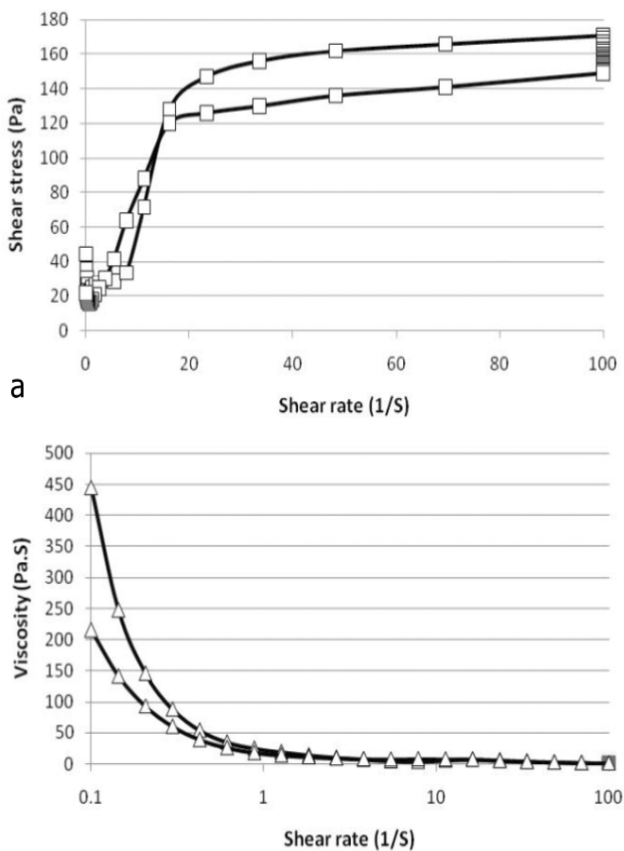


FIGURE 7. THIXOROPY (A) AND VISCOSITY (B) CURVES OF W75P25 PASTE

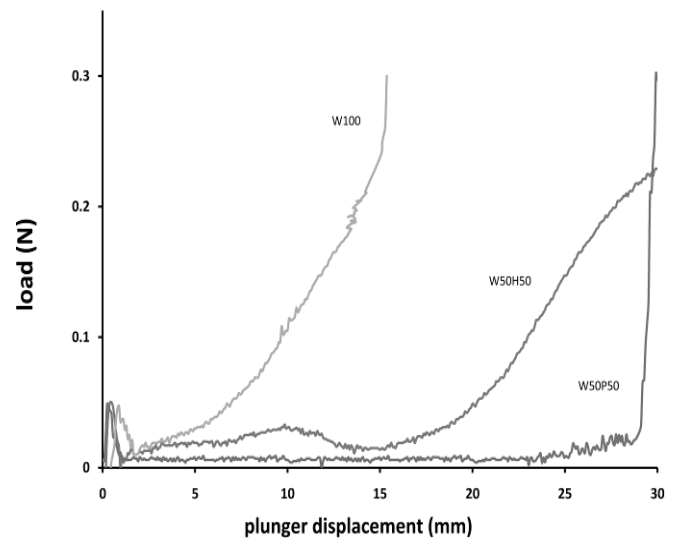


FIGURE 8. THE INJECTION CURVES AS APPLIED FORCE VERSUS PISTON DISPLACEMENT FOR W100, W50H50 AND W50P50 PASTES

Figure 8 shows injectability curves of different CP pastes as applied force versus plunger displacement. For all pastes, the applied force increases suddenly at the beginning of the injection test due to the friction between cylinder wall and paste. In paste prepared with distilled water, displacement of piston increases with applied force up to 15 mm. After 5 mm displacement, when 50 vol% of paste is extruded, the injection process becomes relatively difficult so that no changes in the position of piston occurs by the application of more forces, meaning that the paste cannot be extruded any more. The most important phenomenon during CP paste injection is filter-pressing that occurs through solid-liquid phase separation. Thereafter with higher force, more liquid is extruded and the remaining material in the syringe is not capable to be injected. Increment in liquid phase viscosity of paste (for reducing friction between particles) is a proper strategy to solve filter-pressing problem. As shown in the results, the viscosity of CP paste increases by adding hyaluronic acid and PEG though the injectability has been significantly improved.

In hyaluronic acid-containing paste, after a sharp increase in applied force, the injection continues at a low and constant force, until about 66 vol.% of paste is extruded. After that, a high force region is produced due to the filter-pressing phenomenon and hence the last third of remained paste needs to be extruded by higher force.

In W50P50 paste, the addition of PEG significantly improved the paste injectability so that piston moved

smoothly without filter-pressing and no paste remained in the syringe at the end of the test.

Conclusions

According to the results, following points are concluded:

- CP paste prepared with distilled water has low viscosity but because of its tendency to phase separation during injection, it has improper injectability.
- The presence of hyaluronic acid in paste formulation increases viscosity and thixotropy, leading to improved injectability.
- PEG limits thixotropy of CP paste, increases its viscosity and improves injectability.

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